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Beukeboom, Leo W.; Reed, Kent M.; Werren, John H.

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Effects of deletions on mitotic stability of the Paternal-Sex-Ratio (PSR) chromosome from *Nasonia*

Leo W. Beukeboom*, Kent M. Reed, and John H. Werren

Department of Biology, University of Rochester, Rochester, NY 14627, USA

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Abstract. Paternal-Sex-Ratio (PSR) is a B chromosome that causes all-male offspring in the parasitoid wasp *Nasonia vitripennis*. It is only transmitted via sperm of carrier males and destroys the other paternal chromosomes during the first mitotic division of the fertilized egg. Because of haplodiploidy, the effect of PSR is to convert diploid (female) eggs into haploid eggs that develop into PSR-bearing males. The PSR chromosome was previously found to contain several families of repetitive DNA, which appear to be present in local blocks. PSR chromosomes with irradiation-induced deletions have decreased rates of transmission and increased variation in transmission. This study investigates whether these differences in transmission of deletion chromosomes are due to mitotic instability. Two deletion chromosomes (E306 and F316) and the wild-type PSR chromosome were examined. A cytogenetic assay of testes revealed that wild-type PSR males contained the chromosome in 98%–100% of their spermatocytes. Similar counts from carriers of two deletion chromosomes were lower and varied between individuals from 50%–100%. One F316 male did not contain the chromosome in any of its spermatocytes although the chromosome was present in somatic tissues based on hybridization to PSR-specific repetitive DNA. A molecular analysis of males found the wild-type PSR chromosome to be present in all somatic tissues. Tissue specific differences in the presence of PSR were found in several males from the two deletion lines. The results show that deletions can result in mosaicism due to increased mitotic instability of PSR. Such individuals sometimes partially or completely fail to transmit the chromosome. Patterns of mosaicism of B chromosomes in other organisms are discussed.

* *Present address:* Arbeitsgruppe Michiels, Max-Planck-Institut für Verhaltensphysiologie, D-8130 Seewiesen (Post Starnberg), Federal Republic of Germany

Correspondence to: L. Beukeboom

Introduction

Paternal-Sex-Ratio (PSR) is a supernumerary or B chromosome found in some natural populations of the parasitoid wasp *Nasonia vitripennis* (Werren et al. 1987; Nur et al. 1988; Werren 1991). This wasp has haplodiploid sex determination: males are haploid and develop from unfertilized eggs, whereas females are diploid and develop from fertilized eggs. Normally, PSR is only carried by males and is transmitted via sperm to fertilized eggs. After fertilization of an egg by a PSR-bearing sperm, the paternal chromosomes condense into a chromatin mass, which is subsequently lost, whereas PSR itself survives. The PSR chromosome disrupts normal sex determination by changing fertilized diploid (female) eggs into haploid PSR males and, hence, PSR is exclusively carried by males. PSR is the only known B chromosome of its kind. It is unique in its ability to destroy the complete genome of its carrier each generation. Because of the timing of PSR action, this system may be useful in studying fundamental genetic processes, such as chromosome condensation and replication.

In a molecular analysis PSR was found to contain several tandemly repeated DNA sequences (Nur et al. 1988; Eickbush et al. 1992). Four families were distinguished based upon sequence divergence and lack of cross-hybridization. Repeat families psr2, psr18 and psr22 are specific to PSR, whereas psr79 is also present on the A chromosomes (normal chromosomal complement), but enriched on PSR. The repeats can be used as molecular probes to screen for the presence of PSR (Beukeboom and Werren 1992). Deletion analysis of PSR has shown that most of the repeats are organized in local blocks on the chromosome (Beukeboom and Werren 1993b). Two types of detectable deletion chromosomes were produced. Functional (F) deletion chromosomes are partly or completely missing one or more repeat families, but are still functional and, therefore, are transmitted from males to "sons". Non-functional (NF) deletion chromosomes have functional domains deleted and are transmitted from males to "daughters",

because they no longer destroy the paternal chromosomes in the egg.

Standard (wild-type) PSR chromosomes are transmitted to 94%–100% of fertilized eggs (Beukeboom and Werren 1993a). Thus, PSR males occasionally produce daughters that do not carry PSR. Previous studies have measured transmission rates of several F and NF chromosomes (Beukeboom and Werren in preparation). F chromosomes are transmitted at a high rate through males to sons. NF chromosomes are transmitted at a high rate through males to daughters, but at a low rate through females. Thus, as an unpaired chromosome PSR has high transmission through mitosis (haploid males), but low transmission through meiosis (diploid females).

Most males carrying F-deletion chromosomes have been found to produce daughters at higher frequencies than the wild type (Beukeboom and Werren in preparation), and some males completely failed to transmit the chromosome. These results suggested that PSR is sometimes lost during male development, most likely due to mitotic instability of the chromosome. If correct, this hypothesis predicts that males with such chromosomes will be somatic and germ-line mosaics for PSR. In addition, the level of germ-line mosaicism should be correlated with transmission of the chromosome. We present cytogenetic and molecular data supporting this hypothesis.

Materials and methods

Maintenance of Nasonia. The biology and culturing methods of *Nasonia* have been described in detail (Whiting 1967; Werren 1991; Beukeboom and Werren 1992). Wild-type and PSR-deletion chromosomes were maintained in an MI background (Saul et al. 1965). Further information about creation and maintenance of PSR-deletion lines is presented in Beukeboom and Werren (1993b).

Screening for PSR. Molecular assays using probes of PSR-specific repeats were used to screen for the presence of PSR in adult wasps (Beukeboom and Werren 1992, 1993b). Briefly, in a dot-blot assay, individual wasps are homogenized and dotted onto nitrocellulose filters, which are subsequently hybridized to a PSR-specific probe. Non-carrier males do not show any signal after exposure to autoradiographic film.

Determining mosaicism. Mosaicism for PSR was determined in both the germ line and the soma using different assays.

Cytogenetic assay: testes were prepared from male pupae chosen at random from stock laboratory PSR strains or from hosts taken from diapause stocks. Individual wasp pupae were placed in a drop of *Drosophila* Ringers solution on a microscope slide. Using fine dissecting needles, the testes were removed by rupturing the posterior end of the abdomen and pressing the abdominal contents into the Ringers solution. After removing the Ringers, the testes were fixed in several drops of Carnoy's fixative (3:1 methanol:acetic acid) applied directly to the surface of the slide. Testes were stained with 2.5% Lacmoid's stain (in 1:1:1 H_2O:lactic acid:acetic acid), squashed, and the coverslip sealed with clear nail polish. Prepared slides were stored in the dark at 5° C. Slides were scanned under oil immersion with phase contrast. Metaphases were scored for the presence or absence of the PSR chromosome. Only cells in which all five A chromosomes could clearly be distinguished were scored.

Molecular assay: after reproduction, males carrying an F-deletion chromosome were dissected into head, thorax and abdomen. The head segment was further divided into the head, and left, and right antennae. The thorax segment was divided into the thorax and the left and right forelegs and hindlegs. Middle legs were not separated and wings were discarded because they did not contain sufficient DNA. Each body part was thoroughly homogenized in 30 µl (head, thorax and abdomen) or 10 µl (antennae and legs) homogenization solution (0.2 M NaCl, 0.2 M Tris, 0.02 M EDTA, 2% SDS, pH 7) in a 0.5 ml Eppendorf vial with a glass pestle, similar to preparation of DNA in the dot-blot assay (Beukeboom and Werren 1993b). The pestle was thoroughly cleaned between samples. Resulting solutions were dotted onto two replicate nitrocellulose filters. One filter was hybridized to a mixture of PSR-specific probes (psr10 and psr18), and the other to an A chromosome-specific probe (NV126) (Eickbush et al. 1992; Beukeboom and Werren 1993b). Psr10 and psr18 (as well as psr13 and psr105) belong to the psr18 family and cross-hybridize at normal (65° C and 4 × SSC) stringency. Hybridization to NV126 served as a control for the presence and concentration of DNA in the homogenate. For comparison, homogenates from similar body parts of wild-type PSR-carrying males were dotted on the same filters.

Results

Chromosomes and profiles

Two F-deletion chromosomes (E306 and F316) were chosen for analysis of mosaicism. Both have been maintained for several generations after their creation. The F316 chromosome is the same as previously used in deletion-chromosome pairing studies (Beukeboom and Werren in preparation). Both E306 and F316 completely lack repeats psr10 and psr105, have reduced psr2 and psr22 copy number, but have complete psr13, psr18 and psr79 complements relative to wild-type PSR. These are among the smallest F-deletion chromosomes (Beukeboom unpublished). The deletions of complete repeats are reflected in the approximately 50% reduced size of

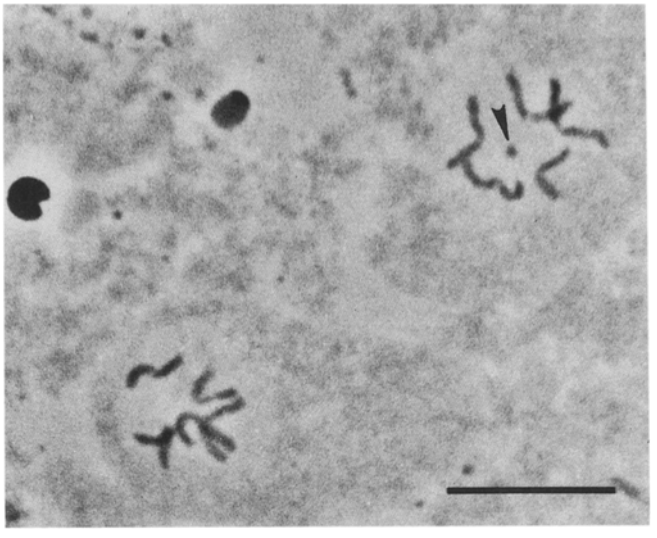

Fig. 1. Two spermatocytes from a male (F316-F) showing testicular mosaicism for the presence of a Paternal-Sex-Ratio (PSR)-deletion chromosome. *Arrowhead* denotes the F316-deletion chromosome in one of the two cells. Bar represents 10 µm

Table 1. Cytogenetic assay for PSR frequency in testes

Male	Total no. of nuclei scored	PSR +	PSR −	%PSR
a. Wild-type PSR males				
A	142	141	1	99.3
B	168	168	0	100.0
C	92	92	0	100.0
D	126	124	2	98.4
E	215	215	0	100.0
F	144	143	1	99.3
G	294	291	3	98.9
H	143	141	2	98.6
I	166	166	0	100.0
J	230	229	1	99.6
K	122	121	1	99.2
L	188	186	2	98.9
M	181	181	0	100.0
N	108	108	0	100.0
Total	2319	2306	13	
b. Deletion-chromosome E306				
A	13	13	0	100.0
B	100	100	0	100.0
C	132	132	0	100.0
D	193	128/47	17/1	90.7
E	107	106	1	99.1
F	74	74	0	100.0
G	58	58	0	100.0
I	37	0	37	0.0
J	160	146	14	91.3
K	6	6	0	100.0
L	237	234	3	98.7
M	106	105	1	99.1
N	155	74	81	47.4
O	34	32	2	94.1
P	175	175	0	100.0
Q	238	0	238	0.0
R	59	59	0	100.0
S	164	164	0	100.0
T	66	66	0	100.0
U	127	127	0	100.0
V	52	52	0	100.0
W	157	156	1	99.4
Total	2450	2054	396	

Table 1 (continued)

Male	Total no. of nuclei scored	PSR +	PSR −	%PSR
c. Deletion-chromosome F316				
F	287	268	19	93.4
I	142	75/65	1/1	98.6
K	233	0	233	0.0
L	154	152	2	98.7
M	247	127/120	1/4	97.9
O	90	88	2	97.8
R	184	179	5	97.3
S	144	123	21	85.4
Y	267	266	1	99.6
Z	223	108/113	2/0	99.1
AA	191	190	1	99.5
BB	205	84/120	0/1	99.5
II	143	100/43	0/0	100.0
JJ	224	223	1	99.5
KK	262	258	4	98.5
LL	75	85/3	75	53.9
MM	141	140	1	99.3
Total	3300	2925	375	

these chromosomes relative to the wild-type PSR chromosome (Fig. 1).

These two chromosomes were chosen because several lines of evidence suggested they were mitotically unstable. First, in extensive genetic studies (Beukeboom and Werren in preparation) carrier males transmitted these chromosomes at frequencies significantly lower than wild type. Occasionally some males did not transmit the chromosome although positive for the chromosome by hybridization (Beukeboom unpublished). Second, molecular profiles of these chromosomes varied between individual carriers in such a way that hybridization intensities were consistently increased or decreased proportionately for every PSR repeat. This suggested that the chromosome was present in tissues of different individuals at varying frequencies (Beukeboom and Werren 1993b).

Germ-line mosaicism

Wild-type PSR. A total of 2319 spermatocytes was scored from 14 PSR pupae for the presence of PSR (Table 1a). The PSR chromosome was found to be present in both testes from each of the 14 males examined. The frequency of PSR in the 14 males ranged from 98.4% to 100.0%. In 6 of the males, all spermatocytes contained the PSR chromosome. However, the remaining 8 males contained at least one spermatocyte lacking PSR.

Deletion-chromosome E306. A total of 2450 spermatocytes from 22 males was scored for the presence of PSR (Table 1b). Although the number of nuclei scored averaged greater than 110 per pupae, the actual number ranged from 6 to 237. This extreme variation was due primarily to differences in the developmental stage of the pupae. Spermatogenesis in *Nasonia* is highly synchronous, with the final cellular divisions occurring within a limited developmental window. Although pupae were prepared on the same day, it was not possible to guarantee that all pupae were at the same developmental stage. Some pupae were clearly more developmentally advanced and thus possessed fewer spermatocytes at metaphase.

PSR was found in testes from 20 of the 22 males (Table 1b). The frequency of PSR in these males ranged from 47.4% to 100.0%. PSR was consistently present

Table 2. Molecular assay and F1 progenies from male carriers of wild-type PSR, and E306- and F316-deletion chromosomes

Chromosome	Individual	Body part									F1 progeny			PSR among F1		
		H	LA	RA	LFL	RFL	LHL	RHL	TH	AB	#♂	#♀	SR	#T	#PSR	pPSR
Wild type	A	+	+	+	+	+	+	+	+	+	106	0	1.0	25	23	0.920
	B	+	+	+	+	+	+	+	+	+	86	0	1.0	25	25	1.0
	C	+	+	+	+	+	+	+	+	+	133	0	1.0			
	D	+	+	+	+	+	+	+	+	+	106	0	1.0			
	E	+	+	+	+	+	+	+	+	+	91	0	1.0			
E306	A	+	+	+	+	−	+	−	+	+	0	75	0			
	B	+	−	+	+	+	+	+	+	+	−	−				
	C	+	+	+	+	+	+	+	+	+	30	26	0.536			
	D	+	+	+	+	+	+	+	+	+	46	36	0.561	30	22	0.733
	E	+	+	+	+	+	+	+	+	+	84	2	0.977			
	F	+	+	+	+	+	+	+	+	+	−	−				
	G	+	+	+	+	+	+	+	+	+	−	−				
	H	+	+	+	+	+	+	+	+	+	−	−				
F316	A	+	+	−	−	−	−	−	−	−	9	64	0.141	9	0	0
	B	+	+	−	+	−	+	+	+	+	1	36	0.027			
	C	+	w	−	w	x	+	w	+	+	12	64	0.158	10	0	0
	D	+	+	w	+	+	+	+	+	+	−	−				
	E	+	+	+	+	+	+	+	+	+	61	5	0.924	30	24	0.800
	F	+	+	+	+	+	+	+	+	+	73	1	0.986			
	G	+	+	+	+	+	+	+	+	+	37	0	1.0			
	H	+	+	+	+	+	+	+	+	+	65	0	1.0	30	22	0.733
	I	+	+	+	+	+	+	+	+	+	−	−				
	J	+	+	+	+	+	+	+	+	+	−	−				

Hybridization intensities of body parts are given for individually dissected males (H, head; LA, left antenna; RA, right antenna; LFL, left foreleg; RFL, right foreleg; LHL, left hindleg; RHL, right hindleg; TH, thorax; AB, abdomen; "+", strong hybridization; "w", weak hybridization; "−", no hybridization). Number of sons (#♂) and daughters (#♀) and the sex ratio (SR, %♂) of their progeny are given. The proportion PSR males among sons was determined for some progenies (#T, number of sons tested; #PSR, number of sons with PSR; pPSR, proportion PSR among sons)

(i.e. 100%) in 12 of the PSR-positive males. Of the remaining 8 males, 4 (E, L, M, and W) had PSR frequencies consistent with the wild-type PSR males (99.1%, 98.7%, 99.1%, and 99.4%, respectively), 3 (D, J, and O) showed slightly lower PSR frequencies (90.7%, 91.3%, and 94.1%, respectively), and in 1 male (N) PSR frequency was 47.4%. One testis from this male was damaged during dissection, and thus, this frequency is representative of only one testis. Cells devoid of the PSR chromosome were generally clustered within the testes. This pattern is indicative of the loss of PSR during a somatic division in stem cells prior to spermatogenesis. Because these males were not molecularly screened, it is not known if PSR was present in the somatic tissues of the 2 males (I and Q) that lacked E306 in their testes. The number of mosaic males (PSR in less than 98% of their spermatocytes) was significantly higher than in the wild-type PSR line (6 out of 22 for E306 versus 0 out of 14 for the wild type; Fisher exact probability test, $P=0.038$).

Deletion-chromosome F316. The F316 chromosome was also used for cytogenetic analysis. Testes were prepared from 40 pupae collected from a single host. Bodies from which the testes had been removed were subsequently dot-blotted to determine the presence of PSR in somatic tissue. Molecular screening showed 17 of the 40 males to be PSR positive (Table 1c). A total of 3300 spermatocytes was scored from the 17 PSR-positive males (range 90–287, mean 194.1). In several preparations, the testes were sufficiently separated on the slide to allow for reliable partitioning of the spermatocytes.

Unlike the E306 males, all but 1 F316 male (II) possessed at least one spermatocyte that lacked the PSR chromosome (Fig. 1, Table 1c). In the majority of the males (12 of 16) the frequency of PSR was reduced only slightly (above 97%). Two males (F and S) showed moderate frequencies (93.4% and 85.4%, respectively). The remaining 2 males were atypical. In 1 male (LL) the PSR frequency was low (53.9%). This individual was found to be mosaic with all but a few of the nuclei containing PSR confined to a single testis. The final male (K) was unique. Although probing positive in the molecular screening of somatic tissue, none of the 233 nuclei examined from this male contained the deletion chromosome. The number of mosaic males was again significantly higher than in the wild type (7 out of 17 for F316 versus 0 out of 14 for the wild type; Fisher exact probability test, $P=0.007$).

Somatic mosaicism

Presence of PSR in separate body parts of individually dissected carrier males was determined by probing homogenates with a "cocktail" of psr10 and psr18 repeats

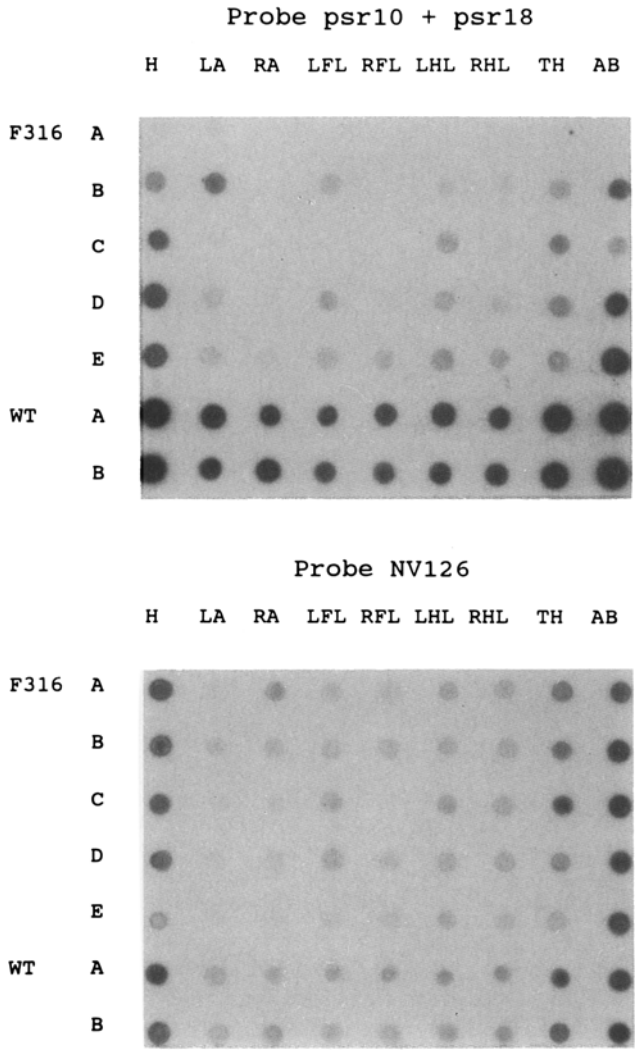

Fig. 2. Dot-blot hybridization results of five F316-carrier males and two wild-type PSR males (*WT*) tested for somatic mosaicism. Presence of PSR is detected on the filter hybridized with a PSR-specific probe (*top*). Presence of DNA in each sample was verified by hybridization to the autosomal NV126 probe (*bottom*). Body parts are as follows: *H*, head; *LA*, left antenna; *RA*, right antenna; *LFL*, left foreleg; *RFL*, right foreleg; *LHL*, left hindleg; *RHL*, right hindleg; *TH*, thorax; *AB*, abdomen

at normal hybridization conditions (65° C and 4 × SSC). Under such conditions this probe hybridizes to each psr18 family repeat. A replicate filter was hybridized to the A-chromosomal NV126 probe for control of DNA load. With one exception (see below), homogenates from all body parts of all individuals, even legs and antennae, showed hybridization on such filters, verifying the presence of DNA in each sample. As expected, larger body parts (i.e. head, thorax and abdomen) generally showed higher hybridization intensities.

Wild-type PSR. Five wild-type males were dissected. All showed consistent hybridization intensities for each body part (Table 2 and Fig. 2, two males shown). All five males produced all-male offspring, indicating 100% transmission of PSR to fertilized eggs. Proportion of PSR among subsequent sons was established for two males (0.92, male A and 1.0, male B, $n=25$ sons tested, Table 2). High PSR frequency among progeny is expected because MI females fertilize 90%–100% of eggs and, therefore, lay few unfertilized eggs that develop into normal (non-PSR) sons.

Deletion-chromosome E306. Two of eight E306-carrier males tested were somatically mosaic (Table 2). One male (A) lacked the chromosome in both its right fore- and hindleg, whereas the other male (B) tested negative only in its left antenna. Presence of DNA in the homogenates of those parts was confirmed with the NV126 probe. Therefore, lack of hybridization was due to absence of E306 in those tissues. Interestingly, male A produced all-female offspring (i.e. no PSR transmission). This suggests that the E306 chromosome was absent in his germ line. Male B did not produce any offspring. Three of the six males (C, D and E) produced high-male sex ratios, indicating partial transmission of PSR, which was confirmed for male D by molecular probing of sons (22 of 30, 73% showed hybridization).

Deletion-chromosome F316. Ten F316-carrier males were tested (Table 2). Results of the molecular assay for five (A–E) males are shown in Fig. 2. In contrast to wild-type PSR males, hybridization intensities of the F316 carriers to the PSR probe varied strongly both between body parts and individuals. For example, within one individual, some parts showed no hybridization, whereas other parts hybridized strongly to PSR (i.e. compare left and right antennae of individual A). On the other hand, hybridization to the NV126 probe did not vary strongly between individuals, indicating consistent DNA content. Hybridization to the NV126 probe verified that all homogenates contained DNA, except the right foreleg of male C.

Four males (A–D) were somatically mosaic. Male A lacked the chromosome in all of its tissues, except head and left antenna, and did not transmit it to any of its male offspring. Male B lacked the chromosome in its right antenna and right foreleg, but not right hindleg. This male also produced progeny with a "normal" female-biased sex ratio. Male C showed no hybridization of its right antenna and right foreleg, weak hybridization of its left antenna, left foreleg, right hindleg, and abdomen, and normal hybridization of the rest of its body parts. Absence of the chromosome in its right foreleg could not be verified, because of lack of hybridization to the NV126 probe. Also, this male did not transmit the chromosome (i.e. female-biased sex ratio and no PSR sons, $n=10$ tested). Male D had weak hybridization of its right antenna, but did not show further mosaicism. He did not produce offspring. Four of the six males (E–H) not found to be mosaic, produced offspring. Two males (G and H) produced all-male progeny, indicating complete transmission of PSR to fertilized eggs. The other two males (E and F) produced a few daughters (5 and 1, respectively, Table 2) indicating incomplete transmission. Frequencies of PSR among sons from male

E were high, as expected. Offspring of male F were not screened for PSR.

Discussion

Previous studies (Werren and Van den Assem 1986; Beukeboom and Werren 1993a) have shown that PSR has high transmission rates through males. Over 90% of wild-type PSR males produced all-male offspring, indicating 100% expression of PSR in fertilized eggs. Most deletion chromosomes had lower transmission rates ranging from 48%–100% (Beukeboom and Werren in preparation). Frequencies of males that either partially transmit or do not transmit the chromosome are higher. Results show that incomplete transmission is due to mitotic instability of PSR resulting in mosaic individuals that carry the chromosome in some tissues, but not in others. Transmission of PSR would then depend on presence of the chromosome in germ-line tissue. Results from this study show that males can indeed be mosaic for PSR, both in their somatic and germ-line tissue.

Germ-line mosaics of B chromosomes have been reported in several species (reviewed in Jones and Rees 1982). Cytogenetic data show that the PSR chromosome is not always present in every spermatocyte. Although absence of PSR in some cells may have been a preparation artifact, the frequencies of wild-type PSR in testes were comparable to transmission rates of wild-type PSR chromosomes. Usually, B-chromosome numbers only vary between individuals, although intra-individual variation has also been reported (reviewed in Jones and Rees 1982). Variation in numbers of B chromosomes between cells may be due to accumulation through non-disjunction or loss through mitotic instability. Accumulation of PSR does not seem to occur, in that it has not been found in more than one copy per cell.

Molecular probing showed that wild-type PSR chromosomes are present throughout the body. Genetic crosses suggested that wild-type PSR chromosomes are sometimes mitotically unstable. PSR loss appears to occur randomly, and thus, differs from some other B chromosomes that undergo selective elimination from somatic tissue (Melander 1950; Hayman et al. 1969; Imai 1974). PSR also contrasts with the only other known case of a B chromosome in Hymenoptera (Imai 1974). In the ant *Leptothorax spinosior*, a B chromosome reportedly occurs at high frequency in male germ-line tissue, but is absent in both male and female somatic tissues.

We have found that some deletions in the PSR chromosome reduce its mitotic stability. Mitotic instability resulted in both somatic and germ-line mosaicism in several individuals. The degree of mosaicism most likely depends on the timing of chromosome loss. Our data suggest that loss can occur at many different stages in development resulting in individuals with different levels of mosaicism. In this study, mosaicism of wild-type PSR was only detected in the germ line (14 individuals tested) and typically at low level. Deletion chromosomes E306 and F316 displayed both somatic and germ-line mosaicism suggesting increased loss rates earlier in development. This is consistent with previous studies, which found their overall transmission rates to be around 0.90 (Beukeboom and Werren in preparation). Many males partially transmitted the chromosome, as expected from the high frequencies of germ-line mosaicism found in this study. It was also shown that males can test positive for the presence of PSR, but lack the chromosome in their testes. This explains why some PSR-positive males do not transmit the chromosome. Occasional failure of wild-type PSR males to transmit the chromosome to any offspring is likely the result of the same process (Beukeboom and Werren 1993a).

Mitotically unstable PSR-deletion chromosomes may be useful for developmental studies, i.e. to construct morphogenetic fate-maps of *Nasonia*, as has been done for *Drosophila* using unstable *X* chromosomes (Hotta and Benzer 1972; Bryant and Zorneter 1973), *Apis* (Milne 1976), and *Habrobracon* (Petters 1977). Clark et al. (1973) found both bilateral and anterior-posterior mosaicism in the parasitoid wasp *Habrobracon*. Although our data are limited, they suggest that both types also occur in *Nasonia*. For example, individuals E306-A and F316-B (Table 2) lacked PSR in some of their right and left body parts, respectively, whereas in F316-A PSR was present in the head, but not thorax and abdomen.

Several factors may be involved in reduced mitotic stability of deletion chromosomes. One is their reduced size. The tandem repeats that comprise the PSR chromosome could serve a stabilizing function (Walker 1971). B chromosomes range in size from minute to as large as or larger than the A chromosomes (Jones and Rees 1982). Although no correlation between size and transmission stability has been reported, a minimum size may be expected for proper behavior at mitosis and meiosis. A second explanation is that absence of specific DNA regions hampers replication of the chromosome and increases the occurrence of breaks in DNA leading to mitotic loss. Little is known about DNA domains that are essential for replication. It has been suggested that certain repetitive DNAs increase recombination (i.e. Jarman and Wells 1989), which involves breakage and reunion of DNA strands (Levin 1987). Therefore, it is possible that deletions in PSR cause aberrant replication resulting in breaks in the chromosome. Structural abnormalities such as ring or dicentric formations (Hotta and Benzer 1972) could also account for decreased mitotic stability of deletion chromosomes; however, these have not been documented in *Nasonia*.

PSR action involves the destruction of all paternal chromosomes while PSR itself survives and is transmitted. Therefore, PSR must somehow be protected against its own action. Conceivably, decreased mitotic stability may also be due to reduced protection of PSR-deletion chromosomes from their own action. At times they may become suicidal by inclusion into the chromatin mass in the early fertilized egg. In transmission studies of deletion chromosomes (Beukeboom and Werren in preparation), a small fraction of males that had apparently been derived from fertilized eggs tested negative for PSR. This indicates that deletion chromosomes are sometimes lost very early in development. Additional cytogenetic stu-

dies of eggs fertilized by sperm carrying F-deletion chromosomes may provide insight into the possible suicidal behavior of deletion chromosomes.

Many B chromosomes have negative effects on the fitness of their carriers (Kimura and Kayano 1961; Hewitt 1973; Robinson and Hewitt 1976; Nur 1966a, b, 1969, 1977) and one may expect selection on the A chromosomes to suppress their effects (Werren et al. 1988). So far, host genetic factors that suppress the drive of a B chromosome have only been demonstrated in an inbred line of maize (Carlson 1969) and in the mealybug *Pseudococcus obscurus* (Nur and Brett 1985, 1987, 1988). In addition, several studies have found differences in B chromosome transmission rates between individuals of a species (Hewitt 1973; Parker et al. 1982; Matthews and Jones 1983; Shaw and Hewitt 1985; Puertas et al. 1990; Viseras et al. 1990). We do not know whether the A chromosomes exercise any effect on the expression or transmission of PSR or its mitotic stability. Because of its detrimental effect (PSR destroys all A chromosomes with which it is associated every generation), chromosomal variants that suppress PSR action should be strongly favored by natural selection (Werren 1987). One possible way of modifying PSR action is to render the chromosome mitotically unstable. Therefore, studies of the effect of genetic background on PSR mitotic stability are warranted.

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